

Interleukin 8 as a vaso-occlusive marker in Brazilian patients with sickle cell disease

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Abstract

Sickle cell disease has a worldwide distribution and is a public health problem in Brazil. Although vaso-occlusive crisis (VOC) is one of the most important clinical features of the disease, there are still several steps of its pathogenesis which are unknown. The increase of the chemotactic factor interleukin 8 (IL-8) has been reported to be involved in sickle cell disease crisis, but this has not been demonstrated conclusively. In the present study we analyzed serum IL-8 levels by ELISA and hematological parameters and hemoglobin patterns by standard techniques in 23 (21 SS and 2 SC) Brazilian patients with sickle cell syndromes during VOC caused by different inducing factors, 22 (21 SS and 1 SC) sickle cell patients out of crisis, and 11 healthy controls. Increased IL-8 levels were observed in 19 of 23 VOC patients (79.2%), 3 of them with more than 1,000 pg/ml. Seventeen of 22 (77.3%) non-crisis patients showed low IL-8 levels (less than 15 pg/ml). Healthy controls had low IL-8 levels. A significant difference in serum IL-8 levels was observed between crisis and non-crisis sickle cell patients ($P < 0.0001$). There was no correlation between IL-8 levels and hematological data or hemoglobin patterns. High serum IL-8 levels were observed in VOC patients independently of the crisis-inducing factor. We conclude that in the studied population, IL-8 concentration may be a useful VOC marker, although the mechanism of the pathogenic process of sickle cell VOC syndromes remains unclear.

Key words

- Interleukin 8
- Sickle cell syndromes
- Vaso-occlusive crisis

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Introduction

Sickling disorders are characterized by the presence of sickle hemoglobin (HbS), which exhibits a point mutation at the sixth codon of the β -globin gene (GAG→GTG), encoding a valine instead of a glutamine in

the β -globin chain. HbS underlies a sickling process described in sickle cell syndromes, such as SS homozygous state and HbSC disease, a doubly heterozygous state represented by two variant hemoglobin products. After deoxygenation, HbS polymers cause a red blood cell (RBC) distortion represented

by cell inflexibility, which is responsible for a variety of cell shapes and consequently for the vaso-occlusive phenomena described in the pathogenesis of the disease (1,2). Sickle cell disease affects millions of people throughout the world and in Brazil the sickle cell trait (AS) occurs with a frequency of 6.9 to 15.4%. The State of Bahia, in the Northeast region of the country, has the highest frequency of abnormal hemoglobin and the highest rate of race admixture, mainly of African origin, causing the presence of hemoglobinopathies to be considered a public health problem (3,4).

Pain is the most common symptom in sickle cell disease. Acute painful cell episodes occur mostly in bone marrow, followed by microvascular occlusion. The long bones and joints are the most common pain sites, but the physiological origin of sickle cell disease is not yet well understood (5). Another clinical feature of sickle cell disease is acute chest syndrome (ACS), which is the most common cause of pulmonary diseases, causing 25% of the deaths of sickle cell disease patients (6,7).

Sickle cell disease has several types of vaso-occlusive pain crises (VOC) such as abdominal and musculoskeletal crises and priapism. The VOC in sickle cell syndromes has been considered to be a process of a complex nature and an important cause of morbidity, which has been associated with several modulating factors, including RBC cell deformity and density, KCl co-transport and Ca^{2+} intravascular concentration, leukocyte and platelet alterations, endothelial cell biology, adherence, hemostatic activation, and reactivity of microvascular tone (8-14).

The role of chemokines, particularly interleukin 8 (IL-8) (15), as a VOC marker has not been well established, especially with respect to the pathogenic mechanism and its property as a VOC risk factor. Polymorphonuclear leukocytes (PMN) may be important as a pathogenic factor during VOC since an

increased number of activated (CD64^+) PMN has been found in VOC sickle cell disease patients (16). The study of several adhesion molecules, such as CD11a, CD11b, CD11c/CD18, L-selectin and CD15, as well as some interleukins such as granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL- α , IL-1, IL-6 and IL-8 described as mediators of PMN binding to endothelium, did not show abnormal expression (15-22). In the present study, we evaluated serum IL-8 levels, hematological parameters and hemoglobin patterns in sickle cell syndrome patients in VOC and compared the results with non-VOC sickle cell disease patients and normal controls.

Subjects and Methods

A total of 23 (21 SS and 2 SC) sickle cell disease patients (10 males and 13 females) presenting VOC due to different inducing factors were studied, including 2 with priapism, 2 with abdominal crisis and 19 with musculoskeletal crisis. Samples were collected before any therapeutic intervention. The two control groups included in the study consisted of 22 (21 SS and 1 SC) sickle cell disease patients out of crisis (10 males and 12 females) and 11 healthy subjects (3 males and 8 females). All patients were from an outpatient clinic of Fundação Hemocentro da Bahia (HEMOBA), Salvador, BA, Brazil, ranging in age from 1 to 45 years. Patients or their guardians gave written informed consent allowing their participation in the study, previously approved by the Institutional Ethics Committee.

Hematological analysis was performed using an electronic cell counter (Coulter Counter T890). Red cell lysates were studied by electrophoresis on cellulose acetate strips at pH 8.4. The presence of HbS was confirmed using a positive sickling and solubility test followed by electrophoresis on agar-citrate, pH 5.3. Hemoglobin A2 was

quantified by elution from cellulose acetate strips after electrophoresis at pH 8.4 and fetal hemoglobin (HbF) levels were measured using an alkali denaturation procedure (23).

The serum IL-8 levels were measured by an enzyme-linked immunosorbent assay (ELISA) (Duoset-Genzyme, Cambridge, MA, USA) according to manufacturer instructions, which considered a level ≤ 15 pg/ml as normal.

Data were analyzed statistically using the EPI Info version 6.0 and Graph Pad Prism software. A P value of less than 0.05 was considered to be statistically significant.

Results

Table 1 shows the hematological parameters of normal and sickle cell syndrome patients in VOC and out of crisis. Data are reported as means \pm SD. There were significant differences between the three groups in HbF ($P = 0.0005$), RBC ($P < 0.001$), white blood cells ($P = 0.0006$), hemoglobin ($P < 0.0001$) and hematocrit ($P < 0.0001$).

The data in Figure 1 indicate a significant increase in IL-8 levels in 19 (82.6%) VOC patients, 3 of them with over 1,000 pg/ml, and 4 (17.4%) VOC patients had IL-8 levels below 15 pg/ml. Seventeen (77.3%) non-crisis patients had low IL-8 levels, and 5 (22.7%) had IL-8 levels over 15 pg/ml (maximum of 87 pg/ml). The healthy control subjects had low IL-8 levels and in some cases, undetectable levels. Figure 1 shows a scattergram of IL-8 level distribution for the three groups studied, showing a statistically significant difference between patients in VOC and out of crisis ($P < 0.0001$, Kruskal-Wallis analysis).

There was no statistically significant correlation between hematological data, hemoglobin pattern and IL-8 levels in this population, even when HbF levels, considered as a prognostic clinical feature of the disease, were taken into account.

Discussion

The results demonstrate that the serum IL-8 levels of most sickle cell syndrome patients in VOC were higher than those of out of crisis patients and healthy controls. We did not find significant differences in hematological parameters or hemoglobin pattern analysis.

High IL-8 levels have been associated with several diseases such as pancreatitis (24), peripheral arterial occlusive disease

Table 1. Hematological parameters of normal subjects and sickle cell syndrome patients in vaso-occlusive crisis (VOC) and out of crisis, including the SS and SC genotypes.

Hematological data	Normal controls (N = 11)	VOC patients (N = 23)	Out of crisis patients (N = 22)
WBC ($\times 10^9/l$)	6.667 \pm 1.326	16.436 \pm 7.128	13.606 \pm 8.0
RBC ($\times 10^{12}/l$)	4.674 \pm 0.321	2.616 \pm 0.670	2.451 \pm 0.526
Hb (g/dl)	13.500 \pm 0.950	7.882 \pm 1.943	7.150 \pm 1.257
Ht (%)	42.080 \pm 2.299	24.965 \pm 6.085	23.267 \pm 3.999
MCV (fl)	90.178 \pm 5.308	93.488 \pm 9.697	96.161 \pm 8.916
MCH (pg)	28.978 \pm 2.101	29.076 \pm 4.159	29.594 \pm 2.901
MCHC (g/l)	32.020 \pm 0.908	30.882 \pm 2.755	30.778 \pm 1.530
HbF (%)	1.364 \pm 0.329	10.772 \pm 8.370	11.741 \pm 7.098

The VOC group consisted of 21 SS and 2 SC patients and the out of crisis patient group consisted of 21 SS and 1 SC patients. The number of normal subjects was 11. $P < 0.05$ is considered significant. WBC, RBC, white and red blood cells, respectively; Hb, hemoglobin; Ht, hematocrit; MCV, MCH, MCHC, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, respectively; HbF, fetal hemoglobin.

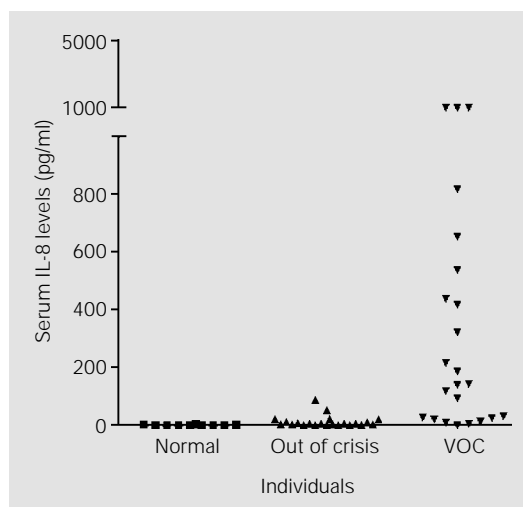


Figure 1. Scattergram showing ELISA analysis of the distribution of interleukin 8 (IL-8) levels in the serum of 23 patients in vaso-occlusive crisis (VOC), 22 out of crisis, and 11 normal healthy subjects. The IL-8 levels of the VOC group were supranormal, while the out of crisis and the normal healthy subject groups had low IL-8 levels ($P < 0.0001$).

(25), endometriosis (26), and myocardial infarction (27). High IL-8 expression has also been attributed to a calcitonin gene-peptide, a neuropeptide with proinflammatory activity in human corneal epithelial cells (28).

High levels of IL-8 during sickle cell disease crisis and during painful crisis have been reported, independently of the crisis-inducing factor (29). High levels of endothelin-1 and prostaglandin-E2 have also been described during painful crises in sickle cell patients in the absence of an increase of other mediators, such as TNF- α , IL- α , IL-6, IL-8 and IL-10 (30). Substance P, known as a TNF- α release stimulating factor and IL-8 promoter, has been reported to be present in high levels in sickle cell disease patients in VOC. The authors found increased IL-8 levels when these were compared to a normal control group, but there was no difference between crisis and out of crisis sickle cell disease patients (31).

High IL-8 levels were reported in serum and bronchoalveolar lavage fluid in a pediatric sickle cell patient group during ACS, suggesting that this cytokine may have a role in focal neutrophil activation (32). A drop in hemoglobin concentration from 1.6 to 2.25 g/dl has been described in adult sickle cell patients with ACS, depending on hemoglobin genotype, as well as increased leukocyte counts of $9.2 \pm 8.3 \times 10^9/l$, and platelet counts of $67 \pm 209 \times 10^9/l$. In the present analysis of hematological and hemoglobin data, we did not find any significant differences between the groups studied, but it is important to state that our series did not include ACS patients (33). Cytokine re-

sponses have been associated with erythropoiesis levels and clinical course of sickle cell disease. Sickle cell disease patients with high HbF levels (>8-9%) had an equilibrium between the inhibitory (TGF- β), the stimulatory stem cell factor (SCF) and IL-3 factors that resulted in a moderate erythropoietic response. Patients with low HbF (<8%) had low levels of TGF- β and an increase of GM-CSF and SCF, maintaining an intense erythropoiesis followed by higher erythropoietic stress (34).

Despite all the controversial reports which reported different levels of the so-called modulating factors in sickle cell disease syndromes, our results demonstrate a significant change in IL-8 levels which was emphasized by the correlation of nonsymptomatic sickle cell patients and healthy controls. On the basis of our results, we conclude that the increased IL-8 levels could be considered as a marker of VOC risk in our population and that the finding of some VOC patients with low IL-8 levels may be related to specific crisis-inducing factors in association with the individual genetic background of each patient, since some genetic inheritance has been associated with the pathogenetic course of the disease (35). Further studies should be conducted in order to determine the exact point before VOC, when serum IL-8 levels begin to increase. This requires the monitoring of the IL-8 levels in sickle cell patients out of crisis, showing how rapidly these levels increase just before VOC development. The ELISA for IL-8 measurement can be performed quickly and can be included in the standard follow-up protocols for these patients.

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